Zinc absorption in women during pregnancy and lactation: a longitudinal study[1–4]

Ellen B Fung, Lorrene D Ritchie, Leslie R Woodhouse, Raimund Roehl, and Janet C King

ABSTRACT Zinc is essential for normal fetal growth and development and for milk production during lactation. The metabolic adjustments made in zinc utilization to meet these needs have not been described. The purpose of this study was to determine whether fractional zinc absorption (FZA) is altered during pregnancy and lactation and, if so, to determine whether the change is related to maternal zinc status, specifically, concentrations of zinc in plasma, erythrocytes, urine, and breast milk and dietary zinc intake. Thirteen women were studied at five time points: once preconception; at 8–10, 24–26, and 34–36 wk gestation; and once while they were lactating 7–9 wk postpartum. Zinc intake increased by 35 μmol/d (2.3 mg/d) from preconception to 34–36 wk ($P = 0.04$); it tended to decrease ($P > 0.05$) during lactation but did not return to the preconception level. The amount of zinc in breast milk averaged 2.0 mg/d at the lactation time point. FZA measured from urinary enrichments of two stable isotopes of zinc increased from 14% preconception to 25% during lactation ($P = 0.023$) but the increase to 19% at 34–36 wk gestation was not significant. No increase in FZA occurred in four women who took iron supplements during lactation. FZA was negatively correlated with plasma zinc concentration at 34–36 wk gestation and with urinary zinc excretion at all time points. The nearly twofold increase in zinc absorption during lactation was presumably in response to the demand for zinc to synthesize breast milk. *Am J Clin Nutr* 1997;66:80–8.

KEY WORDS Zinc absorption, dietary zinc, plasma zinc, breast milk, iron supplementation, pregnancy, lactation, stable isotopes

INTRODUCTION

Zinc is essential for normal embryogenesis and fetal growth. Moderate zinc deficiency during pregnancy has adverse effects on pregnancy outcome in laboratory animals, including altered gestational length, delivery complications, and intrauterine growth retardation (1, 2). Some evidence suggests that zinc deficiency during human pregnancy causes similar complications (3–5) although findings are inconsistent (6, 7). Zinc is also important to the growth of the infant postnatally. Mild zinc deficiency during infancy and early childhood has resulted in stunted growth, decreased midarm muscle circumference, and altered taste acuity (8–10).

The average zinc content in breast milk in the first 3 mo of lactation is 1–2 mg/d. Zinc in breast milk declines with time postpartum; however, most studies have shown no significant difference in zinc content in milk from women who consumed the recommended dietary allowance (RDA) of zinc compared with women who consumed half that amount (11, 12).

In nonpregnant women, the amount of absorbed zinc required to replace endogenous losses is estimated to be 2.5 mg/d (13). This amount is thought to increase to 3.2 mg Zn/d in late pregnancy to support the gain of maternal tissue, amniotic fluid, and fetal growth (14). The requirement for absorbed zinc in the first few months of lactation is greater: ≈4.5 mg/d (14). The third National Health and Nutrition Examination Survey found that the average dietary zinc intake of US women aged 20–40 y is 9.6 mg/d (15). Many studies have shown that women do not alter their zinc intake during pregnancy and the postpartum period (16–18) and that they consume diets in which the amount of zinc is below the RDA for pregnancy (15 mg/d) and lactation (19 mg/d) (13). Healthy nonpregnant women have a great capacity to adjust to varying dietary intakes of zinc. It is not known, however, how pregnant and lactating women adjust utilization of zinc to meet their increased needs.

Intestinal zinc absorption is thought to be the main regulatory site for zinc homeostasis in humans and laboratory animals (19). Assessment of zinc absorption in intestinal segments from laboratory rats showed an 80% increase in pregnant rats and a 133% increase in lactating rats compared with nonpregnant control animals (20). No similar change has been observed in pregnant women (21, 22); preliminary studies of zinc absorption in lactating women (23, 24) yielded conflicting results. In one study in pregnant women and one in lactating women, zinc absorption was measured in the postabsorptive state and, consequently, absorption in all subjects was uncharacteristically high (22, 23). A longitudinal study of changes in absorption of zinc from a meal in women studied from before conception through lactation has not been done. This study is required to

[1] From the Departments of Nutritional Sciences, University of California Berkeley; the California Public Health Foundation, Berkeley; and the Western Human Nutrition Research Center, San Francisco.

[2] Presented in part at the Eighth International Symposium on Trace Elements in Man and Animals, 1993, and Experimental Biology, 1994.

[3] Supported in part by NIH grant 1R01 DK41732 and Johnson, Johnson and Merck, who supplied the prenatal supplements.

[4] Address reprint requests to EB Fung, GI/Nutrition Division, 7th Floor, Children's Hospital of Philadelphia, 34th and Civic Center Boulevard, Philadelphia, PA 19104. E-mail: ebfung@pobox.upenn.edu.

Received September 24, 1996.

Accepted for publication February 18, 1997.

determine whether zinc absorption plays a part in maintaining zinc homeostasis during reproduction in women.

The primary objective of this research, therefore, was to determine whether fractional absorption of a stable isotope of zinc from a standardized meal is altered in well-nourished women followed from before conception through lactation and, if so, to determine whether the change in fractional zinc absorption (FZA) is related to indicators of maternal zinc status, ie, concentrations of zinc in plasma, erythrocytes, urine, and breast milk and dietary zinc intake.

SUBJECTS AND METHODS

Study design and subjects

The protocol for the study was approved by the University of California Berkeley Committee for the Protection of Human Subjects and all participants gave informed written consent. Women who were planning a pregnancy were recruited from the community through newspaper advertisements. An initial screening was done by telephone to identify women (n = 180) who met the following criteria: aged 22–40 y, body mass index (in kg/m^2) of 19–26, nonsmoking, nondiabetic, nonvegetarian, no drug or alcohol abuse, and no previous obstetric or gynecologic complications. Packets containing additional information about the study, a health questionnaire, a 24-h dietary recall form, and a consent form were sent to 85 women who met these criteria. If the women were still interested in participating after reviewing the material, they returned the questionnaire and a signed consent form. A follow-up meeting was then scheduled to review the protocol and answer any questions.

Twenty-six women volunteered for the study and returned signed consent forms; four dropped out for personal reasons before the first measurement. Another subject was dropped from the study after the first time point because of poor compliance. Of the remaining 21 women who were assessed at the first time point, 15 became pregnant; one subject withdrew after the first-trimester measurement and another miscarried. Thus, 13 women (11 whites and 2 Hispanics) completed the entire longitudinal study.

All subjects were assessed at five time points: once before conception; at 8–10, 24–26, and 34–36 wk gestation (first, second, and third trimesters); and once while lactating at 7–9 wk postpartum. FZA was not measured in the first trimester. Each measurement period lasted ≈2 wk. On day 0 of that period, baseline 24-h urine collections were made. On day 1, the test day, isotopic zinc was administered and blood and urine samples were collected. On days 2–11, dietary records and blood and urine samples were collected. To control for variations in hormonal concentrations, the prepregnancy measurement was done in the luteal phase of each woman's menstrual cycle. The luteal phase was determined by a rise in the subject's basal temperature for > 2 d. Day 0 of gestation was defined as the first day of the last menstrual period.

The subjects were given a zinc-free multivitamin-mineral supplement to take daily throughout the study in the morning with breakfast (Stuart formula; Johnson, Johnson & Merck, Fort Washington, PA). Zinc tablets prepared by the University of California San Francisco School of Pharmacy (4.0 mg elemental Zn as zinc sulfate) were taken daily 2 h after dinner from 2 wk before the preconception measurement through the last day of the final postpartum measurement. Four of the 13 subjects also took iron supplements at the midday meal that were prescribed by their physicians. On average, these women took 103 ± 16 mg elemental Fe/d ($\bar{x}$ ± SD) as ferrous sulfate) during the second and third trimesters of pregnancy and 58 ± 4 mg/d during the postpartum measurement. They also received an additional 18 mg elemental Fe/d in the vitamin-mineral supplement.

On the basis of their educational achievements, the participants were considered to have a middle to upper socioeconomic status. About two-thirds of the subjects (n = 9) had completed college and one-third (n = 3) had completed some graduate education. The mean (± SEM) age of the women at the preconception assessment was 30 ± 0.8 y. The average weight and BMI were 60.7 ± 2.0 kg and 22.3 ± 0.8, respectively. Three of the 13 had delivered one baby previously; the remaining 10 were primiparous. The subjects conceived a mean (± SD) of 2.9 ± 3.0 mo after the preconception measurement. Two women (subjects 1 and 16) were pregnant (< 8 wk gestation), as indicated by positive urine pregnancy tests, at their scheduled prepregnancy assessment time. Therefore, the prepregnancy time point for these two subjects was omitted and data obtained at 57 wk postpartum (subject 1) and 67 wk postpartum (subject 16) were used in place of their preconception data. Subject 1 was still partly lactating (less than two infant feedings per day) at this later measurement.

None of the women experienced more than minor problems during their pregnancy; all infants (four females and nine males) were born full-term and healthy. Mean (± SEM) gestational length, maternal weight gain, and birth weights were 39.5 ± 0.4 wk, 15.6 ± 1.2 kg, and 3.65 ± 0.12 kg, respectively.

Sample collection and analyses

During the day before each test day (day 0), the women consumed a standardized diet containing 101 μmol Zn (6.6 mg) and collected a baseline 24-h urine sample in a 4-L polyethylene jug at home. Subjects were instructed to fast between 2200 on day 0 and the next morning (day 1), when they arrived at the metabolic unit in the Department of Nutritional Sciences on the University of California Berkeley campus. Height and weight were measured and an indwelling catheter was placed in an antecubital vein from which a fasting blood sample and all other blood samples were drawn. Blood samples for plasma analyses were collected into Monovettes (02.265.100; Sarstedt, Hayward, CA) containing beads coated with heparin. Hemoglobin and hematocrit were determined in each fasting blood sample with use of standard techniques. Blood samples were kept on ice for a maximum of 2 h, then spun in a clinical centrifuge (International Equipment Co, Needham Heights, MA) at 1400 × g for 10 min at room temperature. Plasma was separated from the red blood cells and samples were placed into separate polypropylene tubes and frozen at − 20°C for later analysis.

FZA was measured by a dual-stable-isotope method at all time points except the first trimester (25). A 45.9-μmol (3.0-mg) dose of ^{68}Zn (as zinc chloride) was dissolved into a nonnutritive beverage (Crystal Lite; Kraft General Foods, Inc, White Plains, NY) and allowed to equilibrate for 24 h. The labeled beverage was given with a standardized breakfast (an English muffin and 24 g peanut butter) at 0800. Breakfast was

consumed within 15 min. Twenty-five minutes after the start of breakfast, 12.2 μmol (0.8 mg) ^{70}Zn (as zinc chloride) was infused into the arm from which blood samples were not drawn. A pregnancy test was done before administration of the isotope on the preconception test day. If the subject was pregnant, intravenous administration of the isotope was not done.

A second 24-h urine collection was begun on the subject's arrival at the metabolic unit on the test day and collection continued through the first morning void on the next day. First spot morning urine samples were then collected until the end of the study, or day 11. All urine samples were weighed and two aliquots were stored at −20°C: a sample acidified to pH 2.0 with concentrated hydrochloric acid and a nonacidified sample. Three-day weighed-food-intake records were completed during the week after each test day.

Isotope preparation

Stable isotopes of zinc were obtained as zinc oxide (Oak Ridge National Laboratory, Oak Ridge, TN). The oral isotope, ^{68}Zn, was prepared by dissolving the labeled zinc oxide in 0.125 mol HCl/L (Seastar Chemicals Inc, Seattle) and adjusting the concentration to 3.0 g Zn/L with triply deionized water. This solution was filtered (Millex-GV 0.22 μm; Millipore, Bedford, MA) and stored at 4 °C. For each subject, 1.0 mL of the ^{68}Zn solution was put into an acid-washed plastic cup and equilibrated for 24 h with 250 mL Crystal Lite beverage.

The intravenous isotope, ^{70}Zn, was prepared by dissolving the labeled zinc oxide into concentrated hydrochloric acid (Seastar Chemicals Inc) and adjusting the concentration to 0.15 g Zn/L with triply deionized water. The pH of the final ^{70}Zn solutions ranged from 2 to 5. The solutions were tested for sterility and pyrogenicity by the University of California San Francisco School of Pharmacy, divided into aliquots, and sealed in sterile 6.0-mL vials.

Dietary intake

The subjects weighed and recorded all food and beverage intake for three complete nonconsecutive days (two weekdays and one weekend day) by using an electronic balance (model 1042-22; Cole-Parmer Inc, Chicago) that was accurate to the nearest 0.1 g. Subjects were given written and oral instructions to record the brand names of food items, to weigh each food item separately, and to avoid foods high in zinc (ie, oysters and zinc-supplemented cereals) during the sample collection period. Nutrient intake was estimated from the food records with use of a computerized nutrient database (NUTRITIONIST III, version 7.2; N-Squared Computing, Salem, OR) that was modified to include zinc values for all foods in the database by using data from Bowes and Church's (26) and the US Department of Agriculture Home Economics Research Report (15).

Measurement of milk output and milk collection

At the lactation time point, the investigator delivered an electronic balance (model LC34; Sartorius, Dublin, CA) with an attached baby seat to each subject's home and set it up for use. Breast-milk output for a 72-h period was determined by weighing the infant on the balance before and after each feeding (27). The balance averages 100 consecutive measurements and is accurate to the nearest 1.0 g. The average milk output for a 24-h period was calculated from the 72-h data. All values were corrected for insensible water loss (28).

After the test weighing procedure (within 1–13 d), milk samples were collected for zinc analysis from each feeding during a 24-h period (27). While the infant breast-fed on one breast, the subject mechanically pumped milk (model 016; Lactina Breast Pump, McHenry, IL) from the other breast into a collection cup. When the infant finished feeding or when no additional milk could be expressed, the subject stopped pumping, mixed the contents of the container cup by inverting it, transferred 10 mL milk to a labeled polypropylene tube, and stored the tube in the freezer. This was done for each feeding in a 24-h period. Milk samples were collected from the subjects' residences and stored at −20 °C. Breast-milk collections were done between 43 and 75 d postpartum.

Erythrocyte lysate preparation

After the plasma was removed from the fasting blood sample obtained on day 1, red blood cells were washed three times with equal volumes of ice-cold 0.9% saline and centrifuged at 1400 × *g* for 15 min at 4 °C; the supernate was discarded. After the last wash, 1–2 mL ice-cold triply deionized water was added to lyse the cells. The lysate was mixed, transferred to a 3.5-mL cryoprecipitation tube, and stored at −20°C until analyzed for zinc and protein concentrations.

Zinc determination

Urine, plasma, and erythrocyte lysate samples were diluted 1:3, 1:8, and 1:20, respectively, with 0.125 mol trace metal—grade hydrochloric acid/L before zinc analysis by atomic absorption spectrometry (model 22; Thermal Jarrell Ash, Franklin, MA). Milk samples were converted to ash overnight in a low-temperature asher (Branson International Plasma Corp, Hayward, CA), diluted with 0.125 mol HCl/L, and analyzed by atomic absorption spectrometry. Zinc standards (range: 0.025–0.3 ppm) were prepared in 0.125 mol HCl/L from a commercially available zinc chloride reference solution (Fisher Scientific, Pittsburgh). A bovine liver standard (batch 1577a; National Institute of Standards and Technology, Gaithersburg, MD) was analyzed with each run as an internal quality control. The CV for the standard averaged 6.5%.

Zinc absorption method

FZA was estimated from the isotopic enrichments of urine samples collected on days 0, 4, 5, and 6 by using a modification of the method of Friel et al (25). Ultrapure acids and bases (Seastar Chemicals Inc) were used in the purification process and for dilution of the samples before analysis by inductively coupled plasma—mass spectrometry (ICP-MS). Approximately 50 mL thawed, acidified urine was centrifuged at 1400 × *g* for 10 min at room temperature to remove solids. The pH of the urine was adjusted to a mean (± SD) of 5.3 ± 0.2 with 3.0 mol NaOH/L and then 70 μL 8.0 mol $NH_4C_2H_3O_2$/L was added. Columns were prepared with 2.0 mL Chelex 100 resin (Bio-Rad Laboratories, Richmond, CA), equilibrated in 2.0 mol HNO_3/L, and rinsed three times with triply deionized water. Columns were converted to the ammonium form by adding 10 mL 2.0 mol NH_4OH/L and rinsing with 30 mL triply deionized water.

The buffered urine was applied to the prepared columns and macrominerals were eluted with 10 mL 1.0 mol $NH_4C_2H_3O_2$/L. The trace elements were eluted with 15 mL 2.5 mol HNO_3/L. The collected fraction was evaporated to dryness in polytetrafluoroethylene beakers, then solubilized in 10 mL 2.5 mol HCl/L and applied to AG1-X8 columns (Bio-Rad Laboratories) (1.0 mL) equilibrated in 2.5 mol HCl/L. The resin was rinsed with 10 mL each of 2.5 and 0.5 mol HCl/L, after which the zinc was eluted off the columns with 0.005 mol HCl/L. The pure zinc fractions were evaporated to dryness in polytetrafluoroethylene beakers on a hot plate. Nitric acid (0.16 mol/L) was added to each sample to reach a final zinc concentration of 0.5 mg/L. The purified samples were analyzed by ICP-MS to obtain isotopic enrichments for ^{68}Zn and ^{70}Zn as described previously (29).

The average values from three spot urine samples collected on days 4, 5, and 6 were used to determine FZA at each time point according to the following equation:

$$FZA(\%) = (^{68}Zn - {}^{68}NA)/(^{70}Zn - {}^{70}NA) \times (^{70}Zn \text{ dose}/^{68}Zn \text{ dose}) \times 100 \quad (1)$$

where ^{68}Zn is ^{68}Zn urinary enrichment, ^{70}Zn is ^{70}Zn urinary enrichment, ^{68}NA is natural abundance of ^{68}Zn [4.57317 (30)], ^{70}Zn is natural abundance of ^{70}Zn [0.15122 (30)], ^{68}Zn dose is the dose of ^{68}Zn in μmol given orally and ^{70}Zn dose is the dose of ^{70}Zn in μmol given intravenously.

Statistical analysis

Statistical outliers were identified by using Dixon's test for outliers (31) and were eliminated from further analysis. Specific outlying values for the dietary data in **Table 1** were a dietary zinc intake of 17.4 mg in subject 21 at the second-trimester assessment and a dietary iron intake of 49.0 mg at preconception and 47.3 mg at the third-trimester assessment in subject 14. Repeated-measures analysis of variance (ANOVA) was conducted with one trial factor—time of study—for analysis of all longitudinal data. Tukey's Student range test was used to identify which time points differed by using a procedure-wise error rate of 5%. The relation between the primary outcome variable (FZA) and other variables was examined with use of repeated-measures analysis of covariance (ANCOVA). This tool provided an assessment of the strength of associations between variables over time. The ANOVA and repeated-measures ANCOVA calculations were done with the SPSS program (SPSS Inc, Chicago). Pearson's correlation coefficients were determined with STATVIEW (version 4.01; Abacus Concepts Inc, Berkeley, CA). Values were considered significant at $P < 0.05$; data are presented as means ± SEMs.

RESULTS

The intake of selected nutrients reported by the subjects in their 3-d weighed-food records is shown in Table 1. Energy intake tended to increase during gestation but the change was not significant. On average, the women consumed 781 kJ more (187 kcal) at the third-trimester than at the preconception assessment. Approximately 53% of energy was consumed as carbohydrate, 32% as fat, and 17% as protein. The source of energy did not change with stage of reproduction. Dietary fiber intake varied from 6.7 to 35.9 g/d and did not change with the study time point.

Total zinc intake (dietary + supplement) averaged 231 ± 4.6 μmol/d (15.1 ± 0.3 mg/d) over the entire study. Dietary zinc intake increased by 23%, or an average of 35 μmol Zn/d (2.3 mg/d), from preconception to the third trimester ($P = 0.04$). Dietary zinc intake at the lactation measurement did not differ significantly from that at the preconception or third-trimester assessment. Because all subjects in the study consumed dairy products regularly, the average preconception calcium intake was ≈27.4 mmol/d (1100 mg/d). Calcium intake increased by 7.5 mmol/d (300 mg/d) from preconception to the third trimester ($P = 0.03$). There was a 35% increase in dietary iron by the third trimester but this trend was not significant. Dietary zinc intake was significantly correlated ($P < 0.05$) with protein intake at preconception ($r = 0.648$) and with both energy intake ($r = 0.602$) and protein intake ($r = 0.838$) at the first-trimester measurement. Dietary zinc and calcium were not correlated at any time point. Dietary zinc and iron were significantly correlated ($P < 0.05$) at preconception, third trimester, and lactation ($r = 0.587$, $r = 0.761$, and $r = 0.698$, respectively).

TABLE 1
Dietary intake of selected nutrients of the subjects[1]

Nutrient	Preconception	Duration of gestation			7–9 wk Postpartum (lactation)
		8–10 wk	24–26 wk	34–36 wk	
Energy (kJ/d)	8782 ± 449	9047 ± 626	8849 ± 550	9563 ± 651	8563 ± 508
Protein (g/d)	83.9 ± 4.4	88.9 ± 5.0	91.1 ± 5.0	93.7 ± 4.8	93.3 ± 5.7
Carbohydrate (g/d)	286 ± 18	284 ± 20	291 ± 20	312 ± 24	233 ± 17
Fat (g/d)	72.1 ± 5.5	78.9 ± 7.2	71.3 ± 6.1	76.1 ± 7.0	74.7 ± 5.8
Fiber (g/d)	20.1 ± 1.5	18.2 ± 2.1	18.2 ± 2.1	22.5 ± 2.0	17.7 ± 1.5
Zinc (mg/d)[2,3]	10.0 ± 0.4	10.0 ± 0.7	11.3 ± 0.6	12.3 ± 0.9[4]	11.2 ± 0.7
Calcium (mg/d)[2]	1102 ± 102	1326 ± 58	1247 ± 72	1401 ± 77[5]	1177 ± 66
Iron (mg/d)[2,3]	13.7 ± 1.2	15.3 ± 1.2	15.4 ± 1.1	18.5 ± 2.2	14.9 ± 1.1

[1] $\bar{x}$ ± SEM; $n = 13$. Values were obtained from the average of 3 d of weighed-food records collected by the subjects and do not include intakes from multivitamin-mineral or zinc supplements.

[2] Daily vitamin-mineral supplements provided an additional 4 mg Zn, 160 mg Ca, and 18 mg Fe; intake from supplements is not included in the data presented.

[3] Because of outlier values for two subjects, the statistical analysis for zinc and iron data were preformed for only 11 subjects.

[4,5] Significantly different from preconception: [4] $P = 0.04$, [5] $P = 0.03$.

Plasma zinc concentration decreased significantly as pregnancy progressed and reached a nadir of 9.58 ± 0.32 μmol/L at 34–36 wk. Plasma zinc concentrations at the second and third trimesters were 22% and 25% lower, respectively, than those at preconception (12.74 ± 0.40 μmol/L). Urinary zinc excretion increased nearly twofold, from 5.66 ± 0.53 to 10.2 ± 1.6 μmol/24 h by the third trimester ($P < 0.05$). Urinary zinc concentration at preconception was not significantly different from that at lactation (8.18 ± 1.39 μmol/24 h).

Of the 13 women, 9 were breast-feeding exclusively at the lactation time point. Total milk output averaged 915 g/d (range: 596–1181 g/d) in those women and was unrelated to week of lactation, infant weight, or number of feedings per day. Total zinc output in milk averaged 26.3 μmol/24 h (1.72 mg/24 h) in all 13 subjects and 33.7 μmol/24 h (2.20 mg/24 h) in the 9 women who were breast-feeding exclusively. Milk zinc output declined with time of lactation because of a decrease in the concentration of zinc in milk. The concentration of zinc in milk was unrelated to infant weight or to maternal indexes of zinc status (ie, plasma, erythrocyte, urinary, or dietary zinc).

FZA increased from 15% before conception to 19% in the second and third trimesters of pregnancy (**Table 2**). This increase was not significant. Given our sample size ($n = 13$), an α of 0.05, and our observed SD, the power to detect a difference at the third trimester was 0.88. Therefore, our inability to detect a significant difference in zinc absorption from preconception to the third trimester was not due to an inadequate sample size. At the lactation time point, FZA averaged 25% and was significantly greater than it was at the preconception measurement ($P = 0.023$).

TABLE 2
Fractional zinc absorption of the subjects[1]

Subject	Preconception	Duration of gestation		7–9 wk Postpartum (lactation)
		24–26 wk	34–36 wk	
01	13.5	16.1	20.7	48.8
04	14.4	12.6	13.5	40.0
05	18.2	22.1	22.1	28.6
07	6.8	7.0	4.1	15.6
12	11.5	13.8	27.4	23.0
13	18.8	31.0	21.7	29.0
14	17.6	35.0	24.7	17.9
15[2]	11.1	9.6	9.7	11.4
16	14.8	14.5	12.4	50.6
17[2]	21.4	26.2	39.7	21.6
18	20.3	21.9	26.9	24.1
20[2]	8.4	21.3	16.9	9.6
21[2]	12.6	14.8	12.8	9.0
$\bar{x}$ ± SEM	14.6 ± 1.3	18.9 ± 2.3	19.4 ± 2.6	25.3 ± 3.9[3]

[1] Calculated from urine samples collected on days 4, 5, and 6.
[2] These subjects were taking iron supplements: subject 15 took 50 mg elemental Fe from 8–10 wk of gestation to 7–9 wk postpartum; subjects 17 took 130 mg from 8–10 wk of gestation to 34–36 wk of gestation and 65 mg from 34–36 wk of gestation to 7–9 wk postpartum; 20 took 130 mg from 24–26 to 34–36 wk of gestation and 50 mg from 34–36 wk of gestation to 7–9 wk postpartum; 21 took 100 mg from 24–26 to 34–36 wk of gestation and 50 mg from 34–36 wk of gestation to 7–9 wk postpartum. All iron was in the form of $FeSO_4$.
[3] Significantly different from preconception, $P < 0.05$.

FZA and plasma zinc concentrations at the third-trimester measurement were negatively correlated ($P < 0.05$, $r = -0.582$). With repeated-measures ANCOVA, FZA was positively correlated with urinary zinc excretion over the entire study ($P < 0.05$). FZA was not correlated with any other variable studied (ie, dietary zinc, energy or protein intake, milk zinc output, or erythrocyte zinc concentration).

FZA in the four women who took iron supplements is compared with that in the nine women who did not take iron supplements in **Figure 1**. FZA did not change significantly during the course of the study in the iron-supplemented group whereas it increased significantly ($P = 0.004$) in the non-iron-supplemented group. At the lactation time point, the women in the non-iron-supplemented group absorbed 31% of the isotopic tracer whereas those in the iron-supplemented group absorbed 13%. Administration of supplemental iron prevented a fall in hemoglobin during gestation and supported higher hemoglobin values at the lactation time point (data not shown). The iron-supplemented and non-iron-supplemented groups had comparable changes in plasma zinc concentrations during the study but urinary zinc excretion did not increase during gestation in the iron-supplemented group whereas it did in the non-iron-supplemented group (**Table 3**). Neither breast-milk volume nor zinc concentrations were affected by iron supplementation (data not shown).

DISCUSSION

This longitudinal study showed that FZA from a standardized breakfast meal was ≈75% greater during lactation than before conception. An upward trend in FZA was also observed during gestation. Because the amount of zinc excreted in breast milk during early lactation is about double that deposited in fetal tissue during late gestation, it is not surprising that the net change in FZA was greater during lactation than during gestation. Although a marked increase in FZA was reported previously in lactating rats (20), this is the first report of an increase

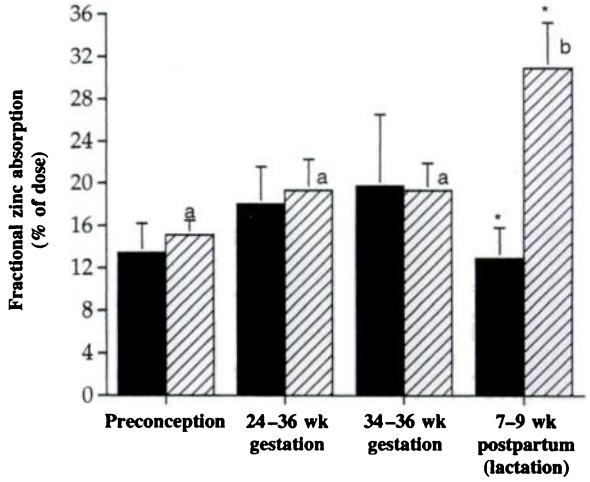

FIGURE 1. Mean (± SEM) fractional zinc absorption from preconception to 7–9 wk postpartum (during lactation) in the iron-supplemented (black bars, $n = 4$) and non-iron-supplemented (hatched bars, $n = 9$) groups. Bars with different letters denote significant differences between time points ($P < 0.05$). *Significantly different from one another ($P = 0.027$).

TABLE 3
Dietary zinc intake, plasma zinc, and urinary zinc excretion in iron-supplemented (n = 4) and non-iron-supplemented (n = 9) subjects[1]

	Duration of gestation			
	Preconception	24–26 wk	34–36 wk	7–9 wk Postpartum (lactation)
Dietary zinc (mg/d)[2]				
Iron supplemented	8.8 ± 0.8	11.6 ± 2.0	12.5 ± 1.0	11.3 ± 1.4
Non-iron supplemented	10.2 ± 0.4	11.2 ± 0.4	12.4 ± 1.1[3]	11.0 ± 0.6
Total iron supplement (mg/d)[4]				
Iron supplemented	18	119 ± 16	119 ± 16	63 ± 13
Non-iron supplemented	18	18	18	18
Plasma zinc (μmol/L)				
Iron supplemented	13.1 ± 0.5	10.2 ± 0.8[5]	10.3 ± 0.6[5]	12.1 ± 1.0
Non-iron supplemented	12.6 ± 0.7	9.8 ± 0.4[6]	9.3 ± 0.4[6]	13.1 ± 0.7
Urinary zinc (μmol/d)				
Iron supplemented	6.2 ± 1.5	7.4 ± 2.5	7.5 ± 2.6	6.7 ± 2.7
Non-iron supplemented	5.4 ± 0.5	8.9 ± 1.3	11.4 ± 1.9[7]	8.8 ± 1.7

[1] $\bar{x}$ ± SEM.
[2] Does not include the 4 mg Zn/d taken by the subjects.
[4] Iron contained in the multivitamin-mineral supplement plus the ferrous sulfate supplement given by the subject's physician.
[3,7] Significantly different from preconception: [3] $P = 0.06$, [7] $P = 0.02$.
[5,6] Significantly different from preconception and 7–9 wk postpartum: [5] $P = 0.009$, [6] $P = 0.0001$.

in a group of women who were followed from before conception to lactation.

Several other studies suggested, however, that zinc absorption increases during lactation in humans. Jackson et al (32) reported that zinc absorption in a group of low-income, lactating Brazilian women was high and ranged from 60% to 85%. This high fractional absorption may have reflected the chronically low zinc intakes of those women (≈128 μmol/d) as well as the increased demand for zinc during lactation. A recent cross-sectional study reported in an abstract provided further evidence that zinc absorption increases during lactation (24). FZA was 83% higher in six lactating women than in seven never-pregnant women. It appears, therefore, that an increase in the intestinal absorption of zinc is one of the mechanisms used to meet the increased demands for zinc during lactation.

A 30% increase in FZA was observed during the second and third trimesters. Although not significant, this slight change suggests that an adjustment in intestinal absorption was one of the mechanisms used to provide the additional zinc needed for fetal growth, perhaps preparatory to onset of the lactation process. It is interesting that FZA was similarly increased in both the second and third trimesters even though the additional need for zinc is thought to be ≈0.6 mg/d in the third trimester and 0.4 mg/d in the second (33). Zinc intake increased in the third trimester. Possibly, a further rise in FZA would have occurred in the third trimester if there had been no increase in zinc intake. An inverse relation between FZA and dietary zinc intake in a group of nonpregnant women was observed previously (34). Alternatively, intestinal zinc absorption may be up-regulated in the second trimester before onset of a period of more rapid fetal growth that normally occurs in the third trimester. Calcium absorption was measured in these same women in a companion study (35). Although the fetal need for calcium is significantly greater in the third than in the second trimester (330 mg/d compared with 50 mg/d), calcium absorption was 49.9% in the second trimester and rose only to 53.8% in the third trimester.

Studies in laboratory animals showed that zinc absorption increases during gestation. Davies and Williams (20) used isolated duodenal loops from pregnant rats to measure zinc absorption in situ at 12, 15, 18, and 21 d of gestation. The rate of zinc absorption on day 21 was twice that in nonpregnant, nonlactating rats. This increase could not be explained by an increase in general absorptive capacity because the absorption of lysine remained constant. In 1981, Scharz et al (36) confirmed these results in studies that used isolated everted intestinal sacs from pregnant rats. Total absorption rose significantly in the last third of pregnancy compared with results in nonpregnant, nonlactating rats. In 1991, Kalinowski and Chavez (37) conducted a study of zinc absorption in pregnant pigs, an animal model that is slightly more representative of human pregnancy. Two groups of pigs were fed diets with different amounts of zinc: a low-zinc diet (10 mg Zn/kg) and a zinc-supplemented diet (50 mg Zn/kg). Zinc absorption was higher and endogenous zinc excretion was lower in the zinc-deficient group. There was no control, nonpregnant group for comparison.

Zinc absorption has also been studied previously in pregnant women. In a balance study, a group of women in the last quarter of pregnancy retained ≈1 mg Zn/d more than that retained by nonpregnant women (38). The difference was not significant. In a cross-sectional study that used a single oral stable isotope of zinc for estimating zinc absorption, no significant difference in zinc absorption between pregnant and nonpregnant women was detected (21). Intraindividual and interindividual variation in zinc absorption may have obviated the differences in zinc absorption in this small (n < 10) cross-sectional study. A study in our laboratory found that interindividual variation in zinc absorption in well-nourished, nonpregnant women consuming a constant diet was ≈25% (39). The variation was higher—≈65%—when the women selected dietary intake freely. Fluctuations in dietary factors and an increase in variability in FZA due to individual physiologic

response may have contributed to the lack of significant changes in zinc absorption during pregnancy observed here.

We used a dual-isotope method to measure FZA in this study. This method was first developed to measure calcium absorption (40) and was adapted for measuring zinc absorption by Friel et al (25). We compared recently the dual-isotope method with the fecal monitoring method that was used extensively for measuring zinc absorption in the past (41). Six nonpregnant, nonlactating women consumed a standardized breakfast containing ^{68}Zn. After the meal they were given a bolus of ^{70}Zn infused for 30 min. FZA from the meal was 33 ± 10%, estimated by the dual-isotope method compared with 38 ± 9% estimated by the fecal monitoring method ($r = 0.94$, $P < 0.01$). These results indicate that the dual-isotope method correlated well with the fecal monitoring method and has the advantage of not requiring complete fecal collections. With the dual-isotope method, FZA can be estimated from plasma, 24-h urine samples, or spot urine samples. If urine samples are used, samples must be collected for ≥ 72 h after the oral dose is taken to ensure complete transport of the oral tracer across the intestinal mucosal (42). If these procedural conditions are met, calculations of FZA by using either plasma, 24-h urine samples, or spot urine samples yield similar results (39).

In this study, FZA was negatively related to plasma zinc concentrations in the third trimester. Several others reported a negative relation between plasma zinc and zinc absorption, suggesting that zinc transport across the mucosal cell is influenced by tissue zinc status (34, 43). Current knowledge suggests that dietary zinc intake, which is often indicative of zinc status, influences zinc absorption by regulating the intestinal concentration of metallothionein (44). A relation between FZA and plasma zinc concentrations during pregnancy was unexpected, however, because the usual expansion in maternal plasma volume causes a drop in plasma zinc concentration. A 25% decline in plasma zinc concentration has been reported by several groups (16, 45, 46). Possibly, a greater decline in plasma zinc concentration signals an increase in intestinal zinc absorption in the presence of a generalized fall in plasma zinc during gestation. In our study, the woman with the greatest decline in plasma zinc concentration (39%) absorbed 27% of zinc whereas the woman with the smallest decline (6.5%) absorbed 4%. Perhaps this decline in plasma zinc reflects an increase in fetal need.

FZA was also positively correlated with urinary zinc excretion within subjects across the entire study when a repeated-measures ANCOVA was used. Because urinary zinc excretion normally rises during pregnancy, presumably because of an increase in glomerular filtration rate (47), a relation with FZA across the entire study was unexpected. Women with increased rates of zinc absorption apparently excreted more urinary zinc during gestation than was accounted for by changes in renal function.

FZA was unrelated to zinc concentrations in breast milk or total secretion of zinc in breast milk in this study even though FZA increased significantly during lactation. This suggests that the concentration of zinc in breast milk is not determined simply by the amount of zinc absorbed. Others have also shown that breast milk zinc is independent of plasma zinc concentration or dietary zinc intake (48). Other body pools of zinc (ie, liver and bone) are perhaps the source of zinc in breast milk. It is plausible to assume that women resorb bone during lactation to accommodate the large calcium requirements of the growing infant (35). From this mobilized bone, zinc also becomes available and may prove to be an important source for breast milk.

Four of the 13 women in this study were prescribed iron supplements (during pregnancy: 102 ± 16 mg elemental Fe/d; during lactation: 58 ± 4 mg elemental Fe/d) by their physicians and the women took these supplements from the second trimester through the lactation assessment time point in the study. Iron supplementation in this small number of women prevented an increase in FZA at the lactation time point, although similar increases in FZA during gestation were observed in the women in the iron-supplemented and non-iron-supplemented groups. It is interesting that zinc absorption was affected during lactation and not in late gestation, when the amount of supplemental iron was higher.

We hypothesize that this difference in zinc absorption was related to shifts in iron absorption during gestation. If nonheme iron and zinc compete for transport proteins into the intestinal cell (49), then the relative abundance of each ion should affect absorption. During gestation, when the need for iron is great, transporters for iron are up-regulated (50) and the uptake of both zinc and iron is accommodated. During lactation, however, erythrocyte iron accumulated during gestation is recycled and intestinal iron absorption is down-regulated. Intake of supplemental iron during lactation may saturate the more limited number of carrier sites and depress zinc uptake.

From these data we cannot conclude that the use of supplemental iron during lactation reduces measures of zinc status. Fasting plasma zinc concentrations did not differ significantly between the women who took iron supplements and those who did not. Urinary zinc excretion, however, did not rise during pregnancy in the iron-supplemented group, as it did in the non-iron-supplemented group, which suggests that less zinc was available for filtering by the kidney and excretion into the urine. These findings are preliminary and further investigations with larger numbers of subjects are warranted to verify the results.

Dietary intake of zinc increased by 35 μmol/d (2.3 mg/d) from preconception to the third trimester. This increase may be explained in part by the increase in consumption of dairy products. Calcium intake increased 7.49 mmol (300 mg) from preconception to the third trimester even though the women were counseled to maintain their usual prepregnancy calcium intake (35). This increase in calcium intake is equivalent to adding 240 mL (1 cup) milk or yogurt/d, which contains 15–31 μmol Zn (1–2 mg).

Average zinc intakes were lower than current recommendations for pregnancy or lactation (13). Total intake of zinc from diet and supplements did not meet the RDA in 10 of the 13 subjects (77%) at any of the time points. Yet, as was shown in previous studies (11, 16, 17, 33), there were no clinical signs of inadequate zinc nutriture, and pregnancy and lactation appeared to be normal. These results support the 1990 recommendation by the Institute of Medicine that 184–214 μmol Zn/d (12–14 mg) from animal protein-based diets is adequate to meet the needs of most pregnant women (51). Similar intakes may also be adequate for lactation. Zinc concentrations in breast milk measured at intervals over the first year postpartum did not vary with dietary zinc intake even though zinc intake ranged from 11 to 28 mg/d (52). Krebs et al (48)

suggested that an intake of 13.0–15.6 mg Zn/d is adequate to meet the increased needs during lactation.

In summary, the well-nourished women in our study met the additional need for zinc during pregnancy by increasing their zinc intake and by a 30% increase in zinc absorption that was not significant. The increase in dietary zinc was due largely to an increase in intake of dairy foods. FZA increased 75% early in the lactation period (7–9 wk postpartum), presumably as an adaptation to the lactation process. We found no such increase in FZA in the few subjects who took iron supplements during lactation. These data indicate that mechanisms regulating zinc homeostasis differ between pregnancy and lactation.

We thank the women in the study for their dedication and patience and Mark Hudes for statistical guidance.

REFERENCES

1. Apgar J. Effect of zinc deprivation from day 12, 15, or 18 gestation of parturition in the rat. J Nutr 1972;102:343–8.
2. Record IR. Zinc deficiency and the developing embryo. Neurotoxicology 1987;8:369–78.
3. Jameson S. Effects of zinc deficiency in human reproduction. Acta Med Scand Suppl 1976;593:1–89.
4. Hambidge KM, Neldner KH, Walravens PA. Zinc, acrodermatitis enteropathica, and congenital malformations. Lancet 1975;1:577–8.
5. Neggers YH, Cutter GR, Acton RT, et al. A positive association between maternal serum zinc concentration and birth weight. Am J Clin Nutr 1990;51:678–84.
6. Campbell-Brown M, Ward RJ, Haines AP, North WRS, Abraham R, McFadyen IR. Zinc and copper in Asian pregnancies: is there evidence for a nutritional deficiency? Br J Obstet Gynaecol 1985;92:875–85.
7. Cherry FF, Sandstead HH, Rojas P, Johnson LK, Batson HK, Wang XB. Adolescent pregnancy: associations among body weight, zinc nutriture, and pregnancy outcome. Am J Clin Nutr 1989;50:945–54.
8. Gibson RS, Vanderkooy PDS, MacDonald AC, Goldman A, Ryan BA, Berry M. A growth-limiting, mild zinc-deficiency syndrome in some Southern Ontario boys with low height percentiles. Am J Clin Nutr 1989;49:1266–73.
9. Hambidge KM, Walravens PA, Brown RM, et al. Zinc nutrition of preschool children in the Denver Head Start program. Am J Clin Nutr 1976;29:734–8.
10. Cavan KR, Gibson RS, Grazioso CF, Isalgue AM, Ruz M, Solomons NW. Growth and body composition of periurban Guatemalan children in relation to zinc status: a longitudinal zinc intervention trial. Am J Clin Nutr 1993;57:344–52.
11. Moser PB, Reynolds RD. Dietary zinc intake and zinc concentrations of plasma, erythrocytes, and breast milk in antepartum and postpartum lactating and nonlactating women: a longitudinal study. Am J Clin Nutr 1983;38:101–8.
12. Moser-Veillon PB, Reynolds RD. A longitudinal study of pyridoxine and zinc supplementation of lactating women. Am J Clin Nutr 1990;52:135–41.
13. National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.
14. King JC, Turnlund JR. Human zinc requirements. In: Mills CF, ed. Zinc in human biology. New York: Springer-Verlag, 1989:335–50.
15. Alaimo K, McDowell MA, Briefel RR, et al. Dietary intake of vitamins, minerals, and fiber of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, phase 1, 1988–91. Atlanta: Centers for Disease Control and Prevention, 1994.
16. Hambidge KM, Krebs NF, Jacobs MA, Favier A, Guyette L, Ikle DN. Zinc nutritional status during pregnancy: a longitudinal study. Am J Clin Nutr 1983;37:429–42.
17. Carbone P, Sobreviela M, Jimenez D, Martinez C, Pocovi M. Hair zinc and dietary zinc intake during pregnancy and puerperium. Eur J Obstet Gynecol Reprod Biol 1992;47:103–8.
18. Breskin MW, Worthington-Roberts BS, Knopp RH, et al. First trimester serum zinc concentrations in human pregnancy. Am J Clin Nutr 1983;38:943–53.
19. Jackson MJ, Lowe NM. Physiological role of zinc. Food Chem 1992;43:233–8.
20. Davies NT, Williams RB. The effect of pregnancy and lactation on the absorption of zinc and lysine by the rat duodenum in situ. Br J Nutr 1977;38:417–23.
21. Swanson CA, Turnlund JR, King JC. Effect of dietary zinc sources and pregnancy on zinc utilization in adult women fed controlled diets. J Nutr 1983;113:2557–67.
22. Hambidge KM, Lloyd L, King JC, et al. Fractional absorption of zinc during pregnancy: a longitudinal study. FASEB J 1992;6:4928 (abstr).
23. Krebs NF, Lloyd L, Westcott JE, et al. Longitudinal study of zinc absorption during lactation. Am J Clin Nutr 1992;56:766 (abstr 67).
24. Moser-Veillon PB, Patterson KY, Veillon C. Zinc absorption is enhanced during lactation. FASEB J 1995;9:A729 (abstr).
25. Friel JK, Naake VL Jr, Miller LV, Fennessey PV, Hambidge KM. The analysis of stable isotopes in urine to determine the fractional absorption of zinc. Am J Clin Nutr 1992;55:473–7.
26. Bowes AP, Church HN. Food values of portions commonly used. 16th ed. Philadelphia: JB Lippincott, 1994.
27. Nommsen LA, Lovelady CA, Heinig MJ, Lonnerdal B, Dewey KG. Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: the DARLING Study. Am J Clin Nutr 1991;53:457–65.
28. Hendrickson EC, Seacat JM, Neville MC. Insensible weight loss in children under one year of age. Acta Paediatr Scand 1985;74:678–80.
29. Roehl R, Gomez J, Woodhouse L. Correction of mass bias drift in ICPMS of zinc isotopic ratios using gallium as an isotope ratio internal standard. J Anal Atomic Spectrom 1995;10:15–23.
30. DeBievre P, Barnes IL. Table of the isotopic composition of the elements as determined by mass spectrometry. Int J Mass Spectrom Ion Proc 1985;65:211–30.
31. Dixon WJ, Massey FJ. Introduction to statistical analysis. 3rd ed. New York: McGraw Hill, 1969.
32. Jackson MJ, Giugliano R, Giugliano LG, Oliveira EF, Shrimpton R. Stable isotope metabolic studies of zinc nutrition in slum-dwelling lactating women in the Amazon valley. Br J Nutr 1988;59:193–203.
33. Swanson CA, King JC. Zinc and pregnancy outcome. Am J Clin Nutr 1987;46:763–71.
34. Morgan PN, Woodhouse L, Serfass RE, Roehl R, King JC. Zinc absorption from a meat and meat free meal in elderly and younger women using stable isotope. FASEB J 1993;7:A279 (abstr 1619).
35. Ritchie LD. Calcium homeostasis during pregnancy, lactation, and following weaning: a longitudinal study of women. PhD thesis. Berkeley: University of California, 1995.
36. Scharz FJ, Kirchgessner M, Sherif SY. Intestinal absorption of zinc during gravidity and lactation. Res Exp Med (Berl) 1981;179:35–42.
37. Kalinowski J, Chavez ER. Metabolic activity of zinc during late pregnancy and lactation of first-litter gilts and their offspring under low dietary zinc intake. J Trace Elem Electrolytes Health Dis 1991;5:187–96.
38. Swanson CA, King JC. Zinc utilization in pregnant and nonpregnant women fed controlled diets providing the zinc RDA. J Nutr 1982;112:697–707.
39. Woodhouse LR, Lowe NM, Schwandt JL, King JC, Gomez J, Roehl R. Validation of a dual isotope method to measure zinc absorption. FASEB J 1995;9:A866 (abstr 5021).
40. Yergey AL, Vieira NE, Covell DG. Direct measurement of dietary fractional absorption using calcium isotopic tracers. Biomed Environ Mass Spectrom 1987;14:603–7.
41. Lowe NM, Woodhouse LR, Schwandt JL, King JC, Gomez J, Roehl R.

Measurement of zinc absorption in humans: a comparison of methods. FASEB J 1995;9:A866 (abstr 5022).
42. Krebs NF, Miller LV, Naake VL, et al. The use of stable isotope techniques to assess zinc metabolism. J Nutr Biochem 1995;6:292–301.
43. Couzy F, Kastenmayer P, Mansourian R, Guinchard S, Munoz-Box R, Dirren H. Zinc absorption in healthy elderly humans and the effect of diet. Am J Clin Nutr 1993;58:690–4.
44. Hempe JM, Cousins RJ. Cysteine-rich intestinal protein and intestinal metallothionein: an inverse relationship as a conceptual model for zinc absorption in rats. J Nutr 1992;122:89–95.
45. Swanson CA, King JC. Reduced serum zinc concentration during pregnancy. Obstet Gynecol 1983;62:313–8.
46. Giroux E, Schechter PJ, Schoun J. Diminished albumin binding of zinc in serum of pregnant women. Clin Sci Mol Med 1976;51:545–9.
47. Hytten F, Chamberlain G. Clinical physiology in obstetrics. Oxford: Blackwell Scientific, 1980.
48. Krebs NF, Reidinger CJ, Hartley S, Robertson AD, Hambidge KM. Zinc supplementation during lactation: effects on maternal status and milk zinc concentrations. Am J Clin Nutr 1995;61:1030–6.
49. Solomons NW. Competitive interaction of iron and zinc in the diet: consequences for human nutrition. J Nutr 1986;116:927–35.
50. Apte SV, Iyengar L. Absorption of dietary iron in pregnancy. Am J Clin Nutr 1970;23:73–7.
51. Institute of Medicine, Committee on Nutritional Status during Pregnancy and Lactation. Nutrition during pregnancy. Washington, DC: National Academy Press, 1990.
52. Kirksey A, Ernst JA, Roepke JL, Tsai T-L. Influence of mineral intake and use of oral contraceptives before pregnancy on the mineral content of human colostrum and of more mature milk. Am J Clin Nutr 1979;32:30–9.